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(54) Title: AN ELECTRONIC TECHNIQUE OF IDENTIFYING AN EFFECTIVE DRUG FOR TREATING A CANCER PATIENT

(57) Abstract

Cancerous cells of a patient under treatment are added to a quantity of cell life supporting media along with a quantity of an anti-cancer drug that is a candidate for treating the patient. The electrical conductivity of the cell is monitored over time in order to determine the effect of the candidate drug to inhibit increases in volume or number of the cancerous cells. Data on the effect of the same drug on normal cells of the patient may simultaneously be gathered so that a drug is chosen which will result in reduced side effects on the patient. A computer system is provided for simultaneously monitoring a large number of media containers, thereby allowing the effects of more than one drug and/or more than one concentration of a given drug to be determined at the same time, within a period of a a few hours or a couple of days.

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AN ELECTRONIC TECHNIQUE OF IDENTIFYING AN EFFECTIVE DRUG FOR TREATING A CANCER PATIENT

Background of the Invention

This invention relates generally to anti-cancer drug therapy and, more specifically, to a technique for selecting the most effective anti-cancer drug to be administered to individual cancer patients.

When designing a cancer chemotherapy treatment program for a particular cancer patient, physicians have a large number of anti-cancer drugs from which to choose. The attending physician's skill and experience is a large factor in the selection of a drug therapy program to attack a particular type of cancer in a patient while at the same time minimizing undesirable side effects of the treatment. Tests are often employed by withdrawing cancerous tissue and observing, in a laboratory, the effect of particular drugs on the increase in the volume and number of cancer cells. The currently utilized techniques require a significant amount of time and are not quantitatively precise.

An electrical quantitative technique for conducting such tests has been proposed by Khan et al., in an article entitled "An Electrical Impedance Method for Rapid Measurement of Tumor Cell Sensitivity to Anti-Cancer Drugs", Drugs Exptl. Clin. Res., VII (5), pages 641-674 (1981). In that technique, a pair of vessels containing suspensions for cancer cells are each provided with two electrodes for passing electrical current through the suspension media. Cancerous cells from a patient are injected into the media of each of

the vessels. An anti-cancer drug being considered for use with the patient is placed in the media of one of the vessels while the other vessel acts as a reference. A 10 KHz. sinusoidal alternating current is passed through each of the cells which are connected in a bridge arrangement. The amount of difference in electrical impedance of the two vessels after a period of time provides an indication of the effectiveness of the drug on the particular cancer cell.

It is a general object of the present invention to provide a technique for determining the effect of specific drugs on changes in volume or number of particular biological cells over time.

It is a more specific object of this invention to provide an improved electronic technique as an aid for selecting the optimum anti-cancer drug for use in treatment of specific cancer patients.

It is another object of this invention to provide such techniques having improved quantitative results and accuracy.

It is a further object of the present invention to provide an electronic system for conveniently and rapidly carrying out such electrical measurements and performing calculations from measured data.

It is yet another object of the present invention to provide equipment and supplies for easy and convenient use in monitoring changes in biological cell volume or number.

Summary of the Invention

These and additional objects are accomplished by the present invention wherein, briefly and generally, the electrical conductivity (or its inverse function, resistivity) of a biological cell life supporting media containing such cells and a drug being tested is

monitored over time. The change in conductivity that is measured is directly related to changes in the volume and number of cells in the media.

According to a more specific aspect of the present invention, the effect of a particular drug on cancerous cells of a medical patient is determined in advance of administering the drug to that patient. Observing the changing conductivity alone provides an accurate, rapid technique for determining the effectiveness of the particular drug to inhibit increases in the volume and number of those cancer cells. It is preferable to monitor this changing impedance by comparison of the impedance of media in another container, as a reference, that contains the same drug but without any of the patient's cancer cells. In a preferred embodiment, an alternating voltage is applied to the electrodes in each of the cells that is very low, less than 1000 Hz., in order to measure the conductivity component of the media's impedance without any significant effects from changing capacitance. A direct current voltage would be preferable for this purpose but that usually results in undesired polarization effects and coating of the electrodes, so a very low frequency alternating current voltage is preferred.

Changing conductivity of the media over time is believed to be directly proportional to increases in the volume and/or number of cells in the media because the concentration of electrically conductive ionic species in the media is believed to be directly related to the cell concentration. A change in conductivity over a period of a few hours or a couple of days, therefore, provides a direct indication of the changing concentration of cells in the media over that time period. The direct relationship between concentration of cells and the measurable ionic species is believed to

be the result of the fact that living cells maintain a constant electrochemical gradient across their boundaries. The electrochemical gradient is determined by the concentration gradient and membrane potential of each ionic species. The electrochemical gradient differs between normal cells and transformed cells. Thus, as the volume of a specific cell increases, it will take in and excrete ions into the media and thereby have a measurable effect upon the conductivity of the media.

According to another aspect of the present invention, normal, non-cancerous cells of the same patient are placed in another container with the same type of media and the same anti-cancerous drug added. Any changing conductivity of the normal cell media is monitored simultaneously with the monitoring of the changing conductivity of the cancer cell media. This allows the physician to determine the effect of the proposed anti-cancer drug on normal cells of the patient and thus provides an indication of the likely level of side effects that will result if that particular drug is applied to the patient. Thus, the physician is provided with enough information for selecting a drug that not only is effective in inhibiting the growth of cancerous cells of the patient but which minimizes its destructive effect on normal, non-cancerous cells of the patient. The techniques of this invention are also useful to monitor the effect of drugs on normal biological cells for other purposes.

According to a further aspect of the present invention, the change in conductivity of a large number of containers with the same media is monitored in order to simultaneously determine the effect of two or more drugs, and/or two or more levels of concentration of the same drug, with both cancerous and normal cells of a patient. In a preferred embodiment, conductivity

readings are taken practically simultaneously of a large number of contained media by a computer system and normalized results provided to show the relative growth of the cancerous cells under the effects of different drugs and/or different drug concentrations, as well as similar effects on the patient's normal cells. By providing all of this information at one time to an attending physician, a fully informed decision can be made as to the best drug or drugs to be administered to the patient.

Additional objects, advantages and features of the present invention will become apparent from the following description of its preferred embodiment, which description should be taken in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1 illustrates generally an overall system for carrying out the various techniques of the present invention;

Figures 2A and 2B are orthogonally oriented cross-sectional views of a single media containing well of the system of Figure 1, taken at sections A-A and B-B, respectively, showing its electrodes;

Figure 3 illustrates details of the electronics of the system of Figure 1;

Figure 4 shows a specific data gathering example use of the system of Figures 1-3;

Figure 5 is a flow chart illustrating an example of the operation of the example system of Figures 1-4 to gather conductivity data for a large number of wells;

Figure 6 is a flow chart illustrating an example of calculations made on the data acquired by performing the steps illustrated in Figure 5; and

Figure 7 illustrates the form of output of information resulting from the calculating steps of Figure 6.

Description of a Preferred Embodiment

A preferred system for conducting the various tests summarized above is first described, primarily with respect to Figures 1-3. A standard, commercially available plastic tissue culture tray 11 has a plurality of identically-shaped wells, such as the well 13, formed in it and open to its top. The tray illustrated is a commonly used 24 well type, but, of course, can be chosen to contain any convenient number of wells. All or a certain desired number of wells are filled with substantially the same quantity of commercially available cell life-supporting media 15. As described in more detail hereinafter, some of the wells also have a drug included in the media. Different concentrations of the same drug may be included in media of different wells, and/or different drugs may be placed in different wells. Biological cells are also included in the media of some of the cells. The purpose of the system being described is to determine the effect of certain drugs in various concentrations upon human or other biological cells, both normal and cancerous.

A standard, commercially available plastic lid 17 is also used with the tray 11. The lid has a flat top with edges protruding downward and normally fits on top of the tray 11. In order to measure the electrical conductivity in each of the wells of the tray 11, a pair of electrodes are provided for each well. A printed circuit board 19 is attached to the top of the lid 17 in order to physically support and provide electrical connection with these electrodes. A pair of electrodes 21 and 23 is provided in the well 13, as illustrated in Figure 2A. Electrodes in the form of pins having a

small circular cross-section are preferred since they minimize the amount of physical disturbance when the electrodes are positioned in the media as a result of the lid 17 being placed onto the tray 11. The electrodes are made to extend downward and close to the bottom of the tray, but to avoid touching it. The pin shaped electrodes also provide a reasonably uniform electrical current distribution between each pair in the media in which they are immersed.

As best seen in Figures 2A and 2B, each of the electrodes penetrates both the lid 17 and the printed circuit board 19. The electrodes are preferably made from stainless steel. A printed copper circuit on the top of the board 19 provides an individual conductor from each of the pins to a connector edge 20 of the board. Examples are conductor traces 25 and 27 connecting with the top end of the electrodes 21 and 23, respectively. Each of the conductive well pins is individually carried through the edge connector 20, a mating socket 29 into which the edge connector is inserted, and by conductors 31 to an electronic data acquisition circuit board 39.

As shown in Figure 1, the tray 11, the lid 17, the printed circuit board 19 attached to the lid 17 and the data acquisition board 39 are all positioned within a commercially available incubator 30. The incubator allows the temperature of the media within the wells of the tray 11 to be carefully controlled. The surrounding atmosphere is also controlled. More than one such tray structure can be included in the incubator at one time for monitoring effects within media of more wells than are available in a single tray. That portion of the circuitry that receives and processes low level electrical signals is placed within the incubator in order to take advantage of the temperature control and electrical shielding it provides and thus reduce

thermally induced measurement errors. After the signals have been amplified to a higher level, they are then coupled to a computer system outside of the incubator through conductors 32.

A computer 33 receives the signal from the data acquisition board 39 through the conductors 32, any necessary interface logic 36, and conductors 34. Appropriate power supply sources 38 and 40 are provided for the circuits within the incubator and any logic 36. The computer 33 is preferably constructed of a standard microcomputer system with a specialized card (board) added to one of its expansion slots. A standard computer system contains a plurality of circuit boards 35, including a standard peripheral input/output board 37, and a special purpose input-output (I/O) board 41. A structure 43 illustrates a common system bus to which all such cards are connected.

The computer system being illustrated for use in the present invention includes the usual peripherals, such as a monitor 49, a keyboard 51 and some type of printer or plotter 53. These are connected to the peripheral input/output board 37 by appropriate cables 55, 57 and 59, respectively. The computer system 33 is illustrated in Figure 3 in three parts. A first part 61 is a basic computer system formed primarily of the cards 35, including a microprocessor 63, system random-access-memory 65, a disk controller 67, and the like, all communicating with each other through the system bus 43 and other circuits (not shown). The disk controller 67 is connected with a disk drive (not shown) by a circuit 69.

A second part of the computer system 33 shown in Figure 3 is the data acquisition board 39 which is connected through the conductors 31 to the electrodes positioned in the media in wells of the tray 11. Well 13 of the tray 11, for example, has its electrode 21

connected to the board 39 while its other electrode 23 is connected to ground potential. One electrode of each well within the incubator 30 is connected to the data acquisition board 39. Its function is to apply a voltage across the electrode pair of each cell within the tray 11 being interrogated, in response to signals in the control circuits 45, and provide in the circuits 47 an analog signal proportional to the current flowing between the electrodes of a connected pair of electrodes.

The third computer component is the measurement input/output board 41, suitable boards being commercially available from several vendors. The board 41, under the control of the microprocessor 63, causes each of the well electrode pairs to be energized, one at a time, and then presents a digital signal on the bus 43 that represents the individual current flow between the energized electrode pair. This data is serially captured by the computer system and appropriately stored in disk memory for later analysis.

The data acquisition board 39 includes a plurality of individual switches, one for each well being monitored, such as the switch 71 that controls connection of the electrodes for the well 13. One such switch is closed at a time. Decoding circuits 73 detect a digital signal in the circuit 45 designating one of the switches and then causes that switch to close so long as the digital signal remains in the control circuits 45.

An alternating voltage source 81 is connected through a series resistance 79 to a common node of all the well switches. The source 81 provides a voltage output that is programmable through the bus 32. An input of an amplifier 83 is also connected to this common node of the well switches. The voltage source 81 is connected in series with a connected well, resulting

in a voltage divider, and the amplifier 83 measures the voltage drop across the connected well. The gain of the amplifier is also programmable through the bus 45 and decoding circuits 75. An output of the amplifier 83 is applied to a sample-and-hold circuit 77 which is also controlled by signals in the bus 32. A signal in the line 47, from an output of the sample-and-hold circuit 77, is passed through the logic circuits 36 to the measurement I/O board, where it is digitized. Since four separate circuits on the data acquisition board 39 are controlled by the bus 35, the bus may be time shared or, preferably, may contain separate conductors extending from the measurement I/O board 41 and logic 36 to each of these controlled circuits.

Operation of the system described with respect to Figures 1-3 in order to carry out the tests summarized above will now be described. A large number of wells within the incubator 30 are utilized for the tests, the specific number depending upon the type and extent of tests to be performed. The same amount of identical cell life supporting media is placed in each of the active wells. The exact media chosen is that which will provide life support to the living biological cells that are being studied and which are placed into the media of at least some of the wells. The media of at least some of the wells also include a drug premixed with the media prior to introduction of the cells. This is the drug whose effect upon the cells is being studied.

Each testing process with the system of Figures 1-3 lasts at least many hours and usually several days. Prior to the beginning of such tests, the biological cells to be studied are plated in the identical media that will be used for the test, without any drug, at least several hours before the test commences. This involves simply allowing the cells to

be studied to equilibrate within the incubator 30 at a uniform temperature and in a rich carbon dioxide atmosphere. At the start of the test, this initial equilibrating media is removed by a standard technique that block the cells and leaves them in the individual wells where they have been equilibrated. The same media is then added back to the well to provide life support to the cells being studied. For those wells where a drug is to be included, the drug is premixed with the media prior to its being placed back in the wells with the cells.

The standard, commercially available tissue culture tray of the type that can be used with the tray 11 includes identically sized wells being about 1.2 cm in diameter with a depth of about 1.5 cm. About 2 ml of media is placed in each well. The number of cells placed in each well is also controlled by standard techniques. Too many cells for a given volume of media results in the cells being starved. Too small a cell density will cause delays in obtaining current readings once a voltage is applied and will also increase the proportion of noise in the electrical measurement being made. For 2 ml of media, a workable range is from 10^4 to 10^6 cells.

In examples of specific testing procedures, 2 ml of Dulbecco's modified minimal essential medium (DMEM) is used in each well, supplemented with 10 percent fetal bovine serum, 200 units of penicillin and 200 ng of streptomycin. About 10^5 human colonic cells are placed in each of the wells that are to contain cells. An FU5 drug is mixed with the media in various wells in three different amounts: 200 nanograms in some wells, 200 micrograms in others, and 2 milligrams in yet others. The tray of wells is maintained in an incubator in an atmosphere containing about seven percent carbon dioxide. The effect of the drug in these various

concentrations on retarding growth of the cancer cells is then measured by the system described herein.

The voltage source 81 is deliberately chosen to have a low frequency, a range of from something above D.C. to something less than 1000 Hz being satisfactory. The frequency is also preferably selected to avoid the 60 Hz line frequency and its harmonics, which can interfere with the readings being taken. A frequency of around 400 Hz has been satisfactorily employed. This frequency range is low enough that any inherent capacitance has little effect since its impedance at the low frequencies is very low. As discussed above, it is desired to measure the conductivity alone without the effects of capacitance so a low alternating frequency voltage source is utilized. The frequency must be high enough, however, to avoid any polarization effects or coating of the electrodes. These effects are substantially eliminated at only a few Hz or higher.

The voltage applied by the source 81 to each pair of electrodes is also carefully digitally controlled. This voltage is made to be less than the voltage generated by biological cells under test that have been placed in the media. That is usually about 90 millivolts. It is desired to keep the voltage as far below that level as possible in order to avoid interfering with life of the cell. If the voltage is made too low, however, the current levels being measured are proportionally low and this can make the signal-to-noise ratio decrease below acceptable limits. A voltage applied across the cell electrodes of about 10 millivolts has been found acceptable for a wide range of applications. The voltage is made as low as possible consistent with the desired signal-to-noise ratio.

Voltage generated by the cells may also contribute to the current reading. For certain types of cells, it may be desirable to provide a reference well

having the same type of media and concentration of cells as the other wells, but without any voltage applied. Electrodes in this reference media would be used to measure the voltage generated by the cells, and this voltage would then be used to compensate for the readings being made in the other cells.

Referring to Figure 4, a specific testing example will be described which involves many aspects of the present invention summarized above, utilizing the system of Figures 1-3 to carry it out. In this example, 60 individual wells are provided, 15 groups of four wells each. Although the same type and volume of media is placed in each of the 60 wells, some will have biological cells, some will have one drug, others another drug, and so forth, in 15 different combinations corresponding to the 15 groups of wells. The four wells in each group are identically constituted, four wells of each type being provided so that an average can be taken to avoid any errors due to inadvertent physical factors. Of course, a different number of wells can be employed in each group, from a single well to ten or more.

Figure 4 is rather self-explanatory. As indicated there, a first group 101 of four wells each contain only the media. No drugs or cells are included in this group. An average of the current readings of each of the four wells of this group 101 is indicated by I1 and is used as a reference with which to compare current readings in other wells.

A second group 103 of four wells contains cancer cells of a medical patient for whom this test is being performed in order to identify the best anti-cancer drug to be administered to the patient. An average of the current readings in each of the four wells of the group 103 is indicated by I2, also used as a reference.

A third group 105 of four wells includes normal cells from the same medical patient within the media. Normal cells are included in this testing example so that the toxicity of each drug on the patient can be determined. The goal is to choose a drug, and a concentration of that drug, which inhibits and preferably stops any increases in cancer cell volume or number while minimizing its negative effect on the viability of normal cells. None of the wells in these first three groups contain any amounts of the drug being tested.

In this Figure 4 example, two different types of drugs are being tested, each drug in two different concentrations. Groups 107, 109, 111 and 113 of wells each include one of the four different drug/concentration combinations in this example. These four groups are also used for reference purposes. Of course, a larger number of different drugs and/or different concentration levels can be simultaneously tested by simply expanding the number of wells that are utilized.

The four groups 115, 117, 119 and 121 of wells contain the same combination of drugs and concentrations as in groups 107, 109, 111 and 113, respectively, but, in this case, each also contain the cancer cells. Similarly, groups of wells 123, 125, 127 and 129 contain the same drug/concentration distribution as in groups 107, 109, 111 and 113, respectively, but each contains the same number of normal cells of the patient.

The raw data of current readings from each of the 60 wells of the example of Figure 4 is acquired by the system of Figures 1-3 in a manner illustrated by the operational flow diagram of Figure 5. Once that data is acquired and stored on the computer system hard disk, it is analyzed and processed in accordance with the flow diagram of Figure 6. The flow diagrams of Figures 5 and

6 represent operation of the system of Figures 1-3 under controlling computer programs.

Before describing the process of Figures 5 and 6 in detail, however, a typical result of the specific example tests of Figure 4 will be described with respect to Figure 7. A curve 131 shows a ratio of the average current I1 (well group 101) divided by the average current I2 (well group 103) over the time of the test. The curve 131 shows the proportional increase in size and number of untreated cancer cells, referenced to current readings through the media only. The goal, of course, is to find a drug and concentration thereof which is close to a straight line, such as that shown in the curve 133, from a ratio of the current average I5 (reference well group 109) to the current average I9 (well group 117). The curve 133 shows very little change over time in the size and number of cancer cells in a media that includes drug no. 1 in concentration no. 2, when normalized with wells having the same media and drug concentration, but no cells. The other curves of Figure 7 illustrate a usual case where other drug/concentration combinations inhibit somewhat increasing size and numbers of the cancerous cells, when compared to the untreated case illustrated by curve 131, but none are as good as that obtained by the drug combination that gives the results given by curve 133. Such an output of the system of Figures 1-3 provides an attending physician with good, easily readable information of the most effective drug/concentration to be prescribed.

A similar type of output can be generated to show the effect of the same drug/concentration combinations on normal cells of the same patient. In this case, a reference curve, showing the effect without any drugs, shows the changes over time of the ratio of average current I1 (reference well group 101) to I3

(well group 105). The effect of the various drugs on normal cells can be shown in comparison by four other curves. One curve shows the change over time of the ratio of average current I4 (well group 107) to I12 (well group 123). Another curve shows the change over the time of the test of the ratio of the average current I5 (well group 109) to I13 (well group 125), and so forth.

Referring to Figure 5, operation of the system of Figures 1-3 to acquire the individual current readings through the media in the various wells is illustrated. A first step 131, in this specific operational example, starts with a first well of a number of wells to be measured. A large number, such as 50, successive measurements of that one cell are taken, each about one second apart.

Each of these 50 or so current measurements for a single well are acquired by the computer system and, as indicated by a step 133, recorded as a single file in disk memory. After the data for one well is obtained, the process asks whether that is the last cell, as indicated by a step 135 of Figure 5. If not, the processor advances to the next well, as indicated by a step 137. That advance is physically accomplished by changing the signal in the circuit 145 to designate a different switch within the board 39 to be closed to connect a new pair of well electrodes to the system. Of course, each of the switches of the board 39, such as the switch 71 for the well 13, is open and closed intermittently the 50 or so times in succession in order to obtain the corresponding 50 or so readings of the well. Once the measurement cycle has advanced to the next well in order at the step 137, another of the switches of the board 39 is operated to make current readings of a different well.

Once all of the wells have been measured this way in sequence, a delay in the measurements, indicated by step 139, occurs. This delay spaces out the frequency of the testing cycle, one such cycle every hour in this specific example. Of course, the frequency can be varied as suitable.

The measurement cycle illustrated in Figure 5 results in a large number of data files being stored in the computer system hard disk, 50 such files being stored every hour in this example. Periodically, it is desired to analyze that raw data and calculate the quantities which can be displayed in some appropriate manner, such as that shown in Figure 7. Figure 6 illustrates such a data processing cycle which can be accomplished once each hour, during the delay 139 in the measurement cycle, or less frequently, depending on how current it is desired to present the processed information.

A first step 141 of the processing cycle calculates an average of each of the 50 measurements taken for a given single well in one measurement cycle. A next step 143 is to calculate from those averages the averages I1, I2, I3, etcetera, for groups of cells.

It can be recognized that there remains a great deal of data, even after the raw data is replaced by these averages. In this example, there are 15 such averages every hour. A typical test may last several days and it is certainly not necessary to show a graph of the type of Figure 7 with data points for every hour over such a long period of time. Thus, as indicated in step 145, it is generally preferable to reduce all of the averages calculated in step 143 to a single set of averages for each group of wells for a day or some other time period suited for the particular test being conducted.

A next step 147 is to calculate from these daily averages the ratios of the type that are displayed in Figure 7. A final step 149 is to add those ratios to a graphical display file in the hard disk for reading out on the monitor or through the printer whenever desired to observe the test results.

For certain types of cancers, there are a limited number of drugs that are known to be potentially effective in their treatment. In these cases, it is most convenient to provide to a clinical laboratory technician a tray having an appropriate media in each well and the various drugs and concentrations in the individual wells from which a test of the type described with respect to Figure 4 can be easily carried out. In this case, the cells are plated in the same media but in a different container for a few hours or a day prior to commencement of the test. The cells are then separated from that initial media and are counted to place substantially the same number of cells in each of the wells. Thus, in order to accomplish the test of Figure 4, a 60 well tray (or multiple trays having a reduced number of wells each) are provided. Twelve of the wells contain only the media. Twelve other of the wells contain drug no. 1 in concentration no. 1, twelve additional wells contain drug no. 1 in concentration no. 2, twelve more wells with drug no. 2 in concentration no. 1, and finally, the last twelve wells with drug no. 2 in concentration no. 2. Of course, additional wells can be provided if more drugs or different concentrations are desired to be included.

In the treatment of malignant cells, the choice of a drug which approaches a zero growth rate, or completely kills the sample, is useful. In addition, if a drug or combination of drugs shows any retarding effect on a particular sample, additional tests might be indicated with higher dosages of that drug or

combination. This would be helpful in determining both the correct dosages and the correct combinations of drugs for a specific tumor.

A quantitative indicator of cellular growth rate would also be of use in clinical and laboratory research. The test will determine the growth rate of a normal cell and it will measure the effect of any stimuli or depressant on a cell's growth rate. This will be useful in studies on toxicity and studies on the effects of growth factors and biological response modifiers on cells. It will also aid in studying and developing substances to enhance the growth rate of biologically engineered strains of antibodies and drugs. The method could also be used to monitor the health of a cell line.

Although the various aspects of the present invention have been described with respect to its preferred embodiment, it will be understood that the invention is entitled to protection within the scope of the appended claims.

IT IS CLAIMED:

1. A method of determining the effect of a particular anti-cancer drug on a cancer patient, comprising the steps of:

placing a large number of cancerous cells from the patient into a quantity of cell life supporting media that contains the anti-cancer drug, and

monitoring any changes over time in electrical conductivity of the media substantially independently of any changing capacitance, whereby changes in current are proportional to the rate of changes in volume and/or number of the cancerous cells and provides an indication of the effect of the anti-cancer drug on such changes.

2. The method according to claim 1 wherein the step of monitoring changes in the media electrical conductivity includes the step of passing through at least a portion of the quantity of media an alternating electrical current that is less than approximately 1000 Hz. in frequency.

3. A method of determining the effect of an anti-cancer drug on a cancer patient, comprising the steps of:

placing a large number of cancerous cells from the patient into a cell life supporting media containing the anti-cancer drug,

positioning a pair of spaced apart pin shaped electrodes into said media,

applying an alternating voltage across said pair of pins that is less than approximately 1000 Hz. in frequency and 90 millivolts in magnitude, and

monitoring any changes over time of electrical current passing through the media between the electrodes, whereby changes in current are proportional

to the rate of growth of the cancerous cells and provides an indication of the effect of the anti-cancer drug to restrain such growth.

4. A method of determining the effect of a particular anti-cancer drug on a cancer patient, comprising the steps of:

monitoring the rate of change of volume and/or number of cancerous cells of the patient in a first quantity of cell life supporting media that contains the anti-cancer drug, and

simultaneously monitoring the viability of non-cancerous cells of the patient in a second quantity of cell life supporting media that contains the anti-cancer drug,

whereby the effect of the particular anti-cancer drug upon normal cells of the patient is determined along with determining the effect of the drug on cancerous cells.

5. The method according to claim 4 wherein the step of monitoring cancerous cell changes includes monitoring electrical conductivity of the first quantity of media, and wherein the step of monitoring the viability of non-cancerous cell includes monitoring electrical conductivity of the second quantity of media.

6. A method of determining the effect of an anti-cancer drug on a cancer patient, comprising the steps of:

monitoring a magnitude of electrical conductivity in a volume of a quantity of cell life supporting media that contains both cancerous cells of the patient and the anti-cancer drug,

simultaneously monitoring the magnitude of electrical conductivity in a volume of a separate

quantity of cell life supporting media that contains the anti-cancer drug without any cells of the patient, and

comparing any changes of electrical conductivity, whereby the effect of the anti-cancer drug upon cancerous cells of the patient is determined.

7. A method of identifying an effective anti-cancer drug for use in treating a cancer patient, comprising the steps of:

monitoring changes in volume and/or number of cancerous cells of the patient in a first quantity of cell life supporting media that contains a first anti-cancer drug,

simultaneously monitoring changes in volume and/or number of cancerous cells of the patient in a second quantity of cell life supporting media that contains a second anti-cancer drug, and

comparing the monitored changes, whereby the most effective of the first and second anti-cancer drugs in restraining growth of cancerous cells of the patient is determined.

8. The method according to claim 7 which comprises the additional steps of:

monitoring the rate of changes in volume and/or number of non-cancerous cells of the patient in a third quantity of cell life supporting media that contains said first anti-cancer drug,

simultaneously monitoring the viability of non-cancerous cells of the patient in a fourth quantity of cell life supporting media that contains said second anti-cancer drug, and

whereby the effect of the first and second anti-cancer drugs upon normal cells of the patient is determined as well as determining the effect of the drug on cancerous cells.

9. The method according to claim 7 wherein the step of monitoring changes in the first quantity of media includes monitoring an electrical conductivity of the first quantity of media, and wherein the step of monitoring changes in the second quantity of media includes monitoring an electrical conductivity of the second quantity of media.

10. A method of determining the effect of anti-cancer drugs on a cancer patient, comprising the steps of:

establishing a plurality of containers in at least two groups having at least one container in each group, said containers holding a quantity of cell life supporting media having the anti-cancer drug therein,

placing a large number of cancerous cells from the patient into media of the containers of the first group but not those of the second group,

applying a voltage across the media in the containers of the first and second groups and individually monitoring the magnitude of a resulting electrical current therethrough,

averaging the current flow in the containers of the first group,

averaging the current flow in the containers of the second group,

comparing the average current flow of the containers of the first and second group, and

monitoring any changes over time in the comparison, whereby changes are proportional to the rate of growth of the cancerous cells and provides an indication of the effect of the anti-cancer drug to restrain such growth.

11. The method of claim 10, comprising the additional steps of:

establishing a third group of containers having at least one container therein and holding a quantity of cell life supporting media and the anti-cancer drug therein,

placing a large number of non-cancerous cells from the patient into media of the containers of the third group but not in those of the first or second group,

applying a voltage across the media in the containers of the third group and individually monitoring the magnitude of a resulting electrical current therethrough,

averaging the current flow in the containers of the third group,

comparing the average current flow of the containers of the second and third group,

monitoring any changes over time in the comparison, whereby changes are proportional to the effect of the anti-cancer drug upon normal cell replacement in the patient.

12. The method of claim 10, comprising the additional steps of:

establishing a third and a fourth group of containers having at least one container in each group, said containers holding a quantity of cell life supporting media therein,

placing a large number of cancerous cells from the patient into media of the containers of the third group but not in those of the fourth group, the media of the containers of the third and fourth groups not receiving the anti-cancer drug,

applying a voltage across the media in the containers of the third and fourth groups and individually monitoring the magnitude of a resulting electrical current therethrough,

averaging the current flow in the containers of the third group,

averaging the current flow in the containers of the fourth group,

comparing the average current flow of the containers of the third and fourth group, and

monitoring any changes over time in the comparison, whereby changes are proportional to the degree of unrestrained changes in the volume and/or number of cancer cells in said media.

13. The method of claim 10, comprising the additional steps of:

establishing a third, a fourth and a fifth group of containers having at least one container in each group, said containers holding a quantity of cell life supporting media therein and without the anti-cancer drug,

placing a large number of cancerous cells from the patient into media of the containers of the third group but not in those of the fourth or fifth group,

placing a large number of non-cancerous cells from the patient into media of the containers of the fourth group but not in those of the third or fifth group,

applying a voltage across the media in the containers of the third, fourth and fifth groups and individually monitoring the magnitude of a resulting electrical current therethrough,

averaging the current flow in the containers of the third group,

averaging the current flow in the containers of the fourth group,

averaging the current flow in the containers of the fifth group,

comparing the average current flow of the containers of the third and fifth group, and

comparing the average current flow of the containers of the fourth and fifth group, and

monitoring any changes over time in the comparisons, whereby changes are respectively proportional to the degree of unrestrained changes in the volume and/or number of cancer cells in said media and to the degree of unrestrained changes in the volume and/or number of normal cells in said media.

14. A method of determining the effect of anti-cancer drugs on a cancer patient, comprising the steps of:

establishing a plurality of containers in at least two groups having at least one container in each group, said containers holding a quantity of cell life supporting media and the anti-cancer drug,

placing a large number of cancerous cells from the patient into media of the containers of the first group but not in those of the second group,

placing a large number of non-cancerous cells from the patient into media of the containers of the second group but not in those of the first group,

applying a voltage across the media in the containers of the first and second groups and individually monitoring the magnitude of a resulting electrical current therethrough,

averaging the current flow in the containers of the first group,

averaging the current flow in the containers of the second group, and

monitoring any changes over time in the average current flows, whereby changes in the current flow in the containers of the first group are proportional to changes in the cancerous cells and

provides an indication of the effect of the anti-cancer drug, and whereby changes in the current flow in the containers of the second group are proportional to changes in the non-cancerous cells and provides an indication of the effect of the anti-cancer drug upon normal cells of the patient.

15. A tray having a plurality of individual wells therein, wherein a first group of at least one well contains a cell life supporting media containing a first anti-cancer drug, a second group of at least one well contains a cell life supporting media containing a second anti-cancer drug, and a third group of at least one well contains a cell life supporting media substantially without any anti-cancer drug therein.

16. A lid adapted for use with a tray of a plurality of wells, comprising:

a cover adapted to be positioned on said tray in a manner to cover its said wells,

a printed circuit board attached to a top surface of said cover,

a plurality of pairs of electrically conductive pins attached at one end to said printed circuit board and extending through holes in said cover, each pair being positioned to extend into a different one of at least a portion of said tray wells, whereby the pins electrically contact media within the wells, and

a plurality of electrically conductive traces formed on a surface of said printed circuit board and extending from said pins to an edge of said board, whereby the pins may be electrically connected through an edge of the board.

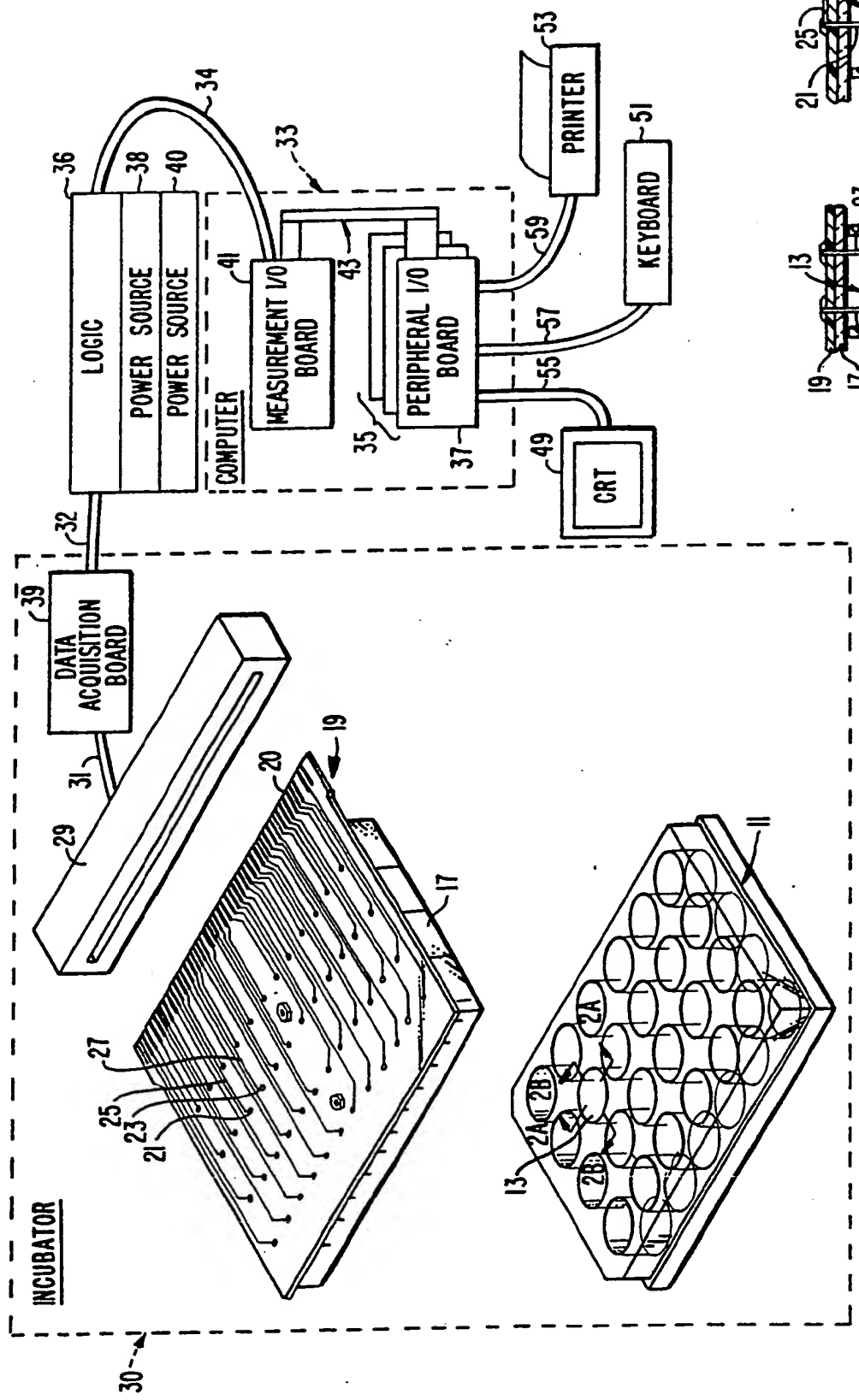

FIG. 1.

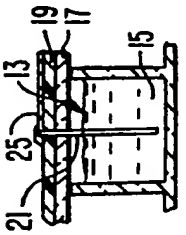

FIG. 2B.

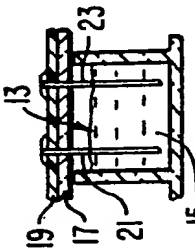

FIG. 2A.

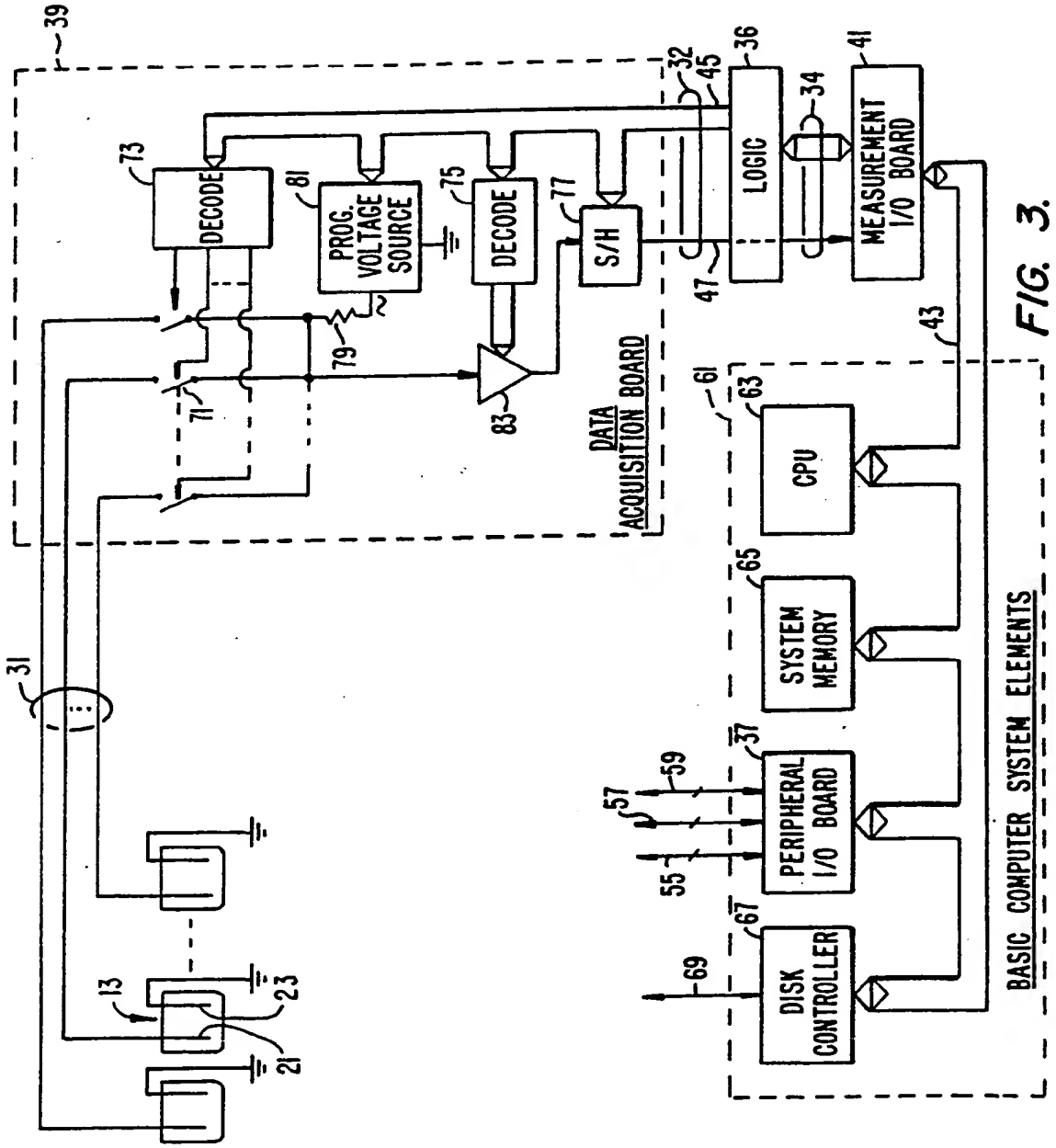
39
DECODE
73
PROG. VOLTAGE SOURCE
81
DECODE
75
S/H
77
LOGIC
36
MEASUREMENT I/O BOARD
41
32
45
34
47
79
71
83
DATA ACQUISITION BOARD
43
61
63
CPU
65
SYSTEM MEMORY
37
PERIPHERAL I/O BOARD
57
59
55
67
DISK CONTROLLER
69
BASIC COMPUTER SYSTEM ELEMENTS
31
13
23
21

FIG. 3.

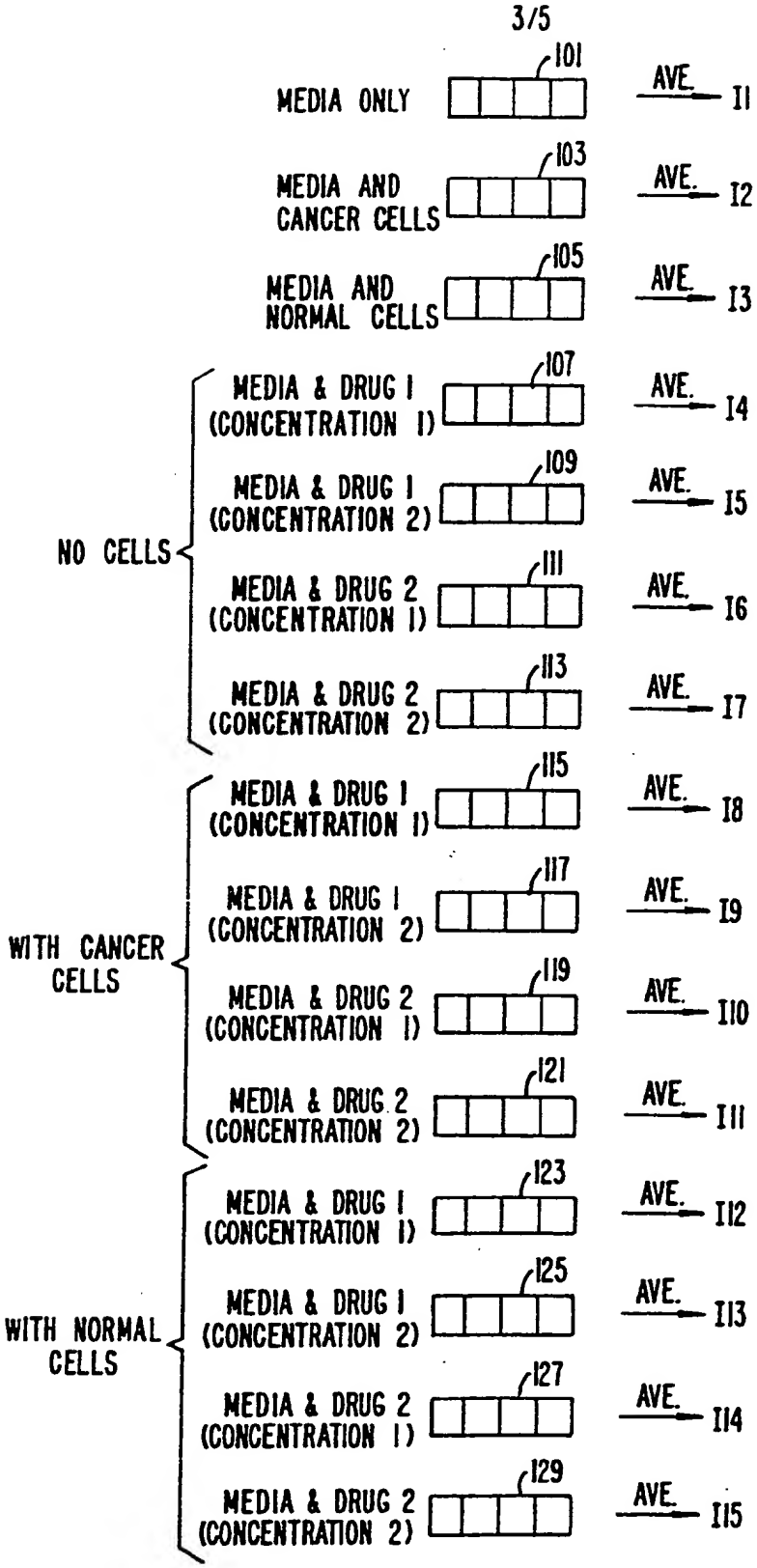
MEDIA ONLY
101
AVE. I1
MEDIA AND CANCER CELLS
103
AVE. I2
MEDIA AND NORMAL CELLS
105
AVE. I3
NO CELLS
MEDIA & DRUG 1 (CONCENTRATION 1)
107
AVE. I4
MEDIA & DRUG 1 (CONCENTRATION 2)
109
AVE. I5
MEDIA & DRUG 2 (CONCENTRATION 1)
111
AVE. I6
MEDIA & DRUG 2 (CONCENTRATION 2)
113
AVE. I7
WITH CANCER CELLS
MEDIA & DRUG 1 (CONCENTRATION 1)
115
AVE. I8
MEDIA & DRUG 1 (CONCENTRATION 2)
117
AVE. I9
MEDIA & DRUG 2 (CONCENTRATION 1)
119
AVE. I10
MEDIA & DRUG 2 (CONCENTRATION 2)
121
AVE. I11
WITH NORMAL CELLS
MEDIA & DRUG 1 (CONCENTRATION 1)
123
AVE. I12
MEDIA & DRUG 1 (CONCENTRATION 2)
125
AVE. I13
MEDIA & DRUG 2 (CONCENTRATION 1)
127
AVE. I14
MEDIA & DRUG 2 (CONCENTRATION 2)
129
AVE. I15

FIG. 4.

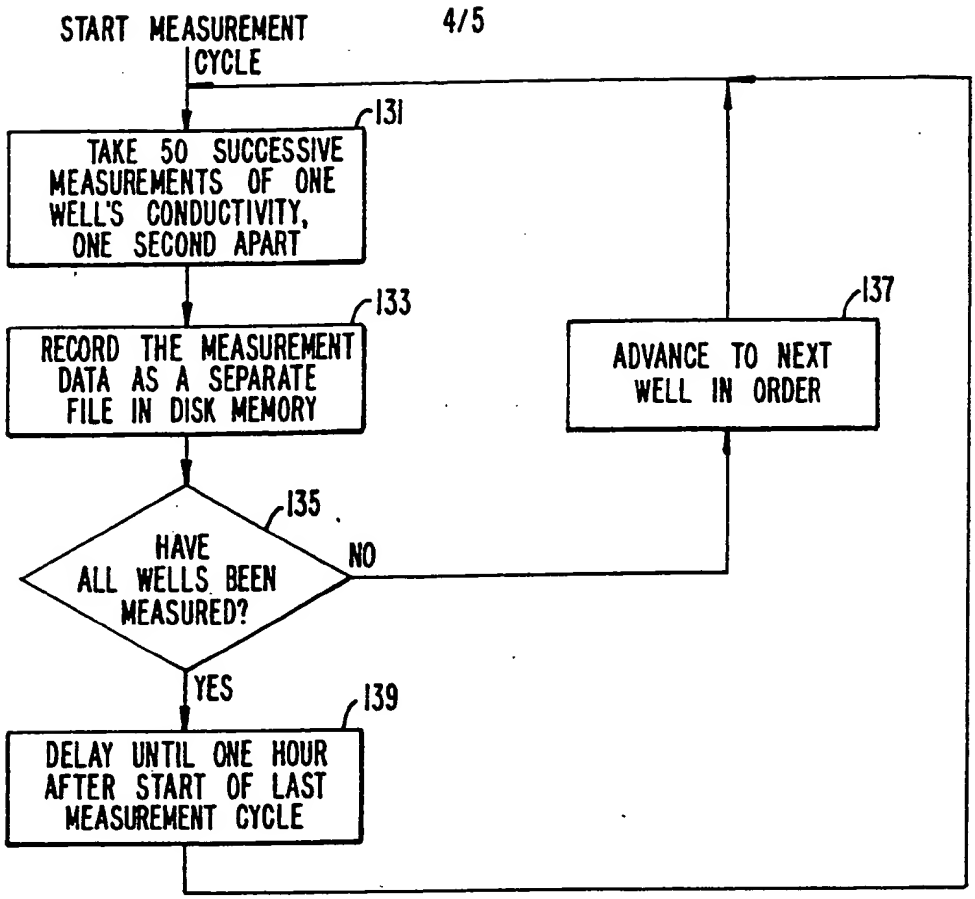
START MEASUREMENT CYCLE
131
TAKE 50 SUCCESSIVE MEASUREMENTS OF ONE WELL'S CONDUCTIVITY, ONE SECOND APART
133
RECORD THE MEASUREMENT DATA AS A SEPARATE FILE IN DISK MEMORY
135
HAVE ALL WELLS BEEN MEASURED?
NO
YES
137
ADVANCE TO NEXT WELL IN ORDER
139
DELAY UNTIL ONE HOUR AFTER START OF LAST MEASUREMENT CYCLE

FIG. 5.

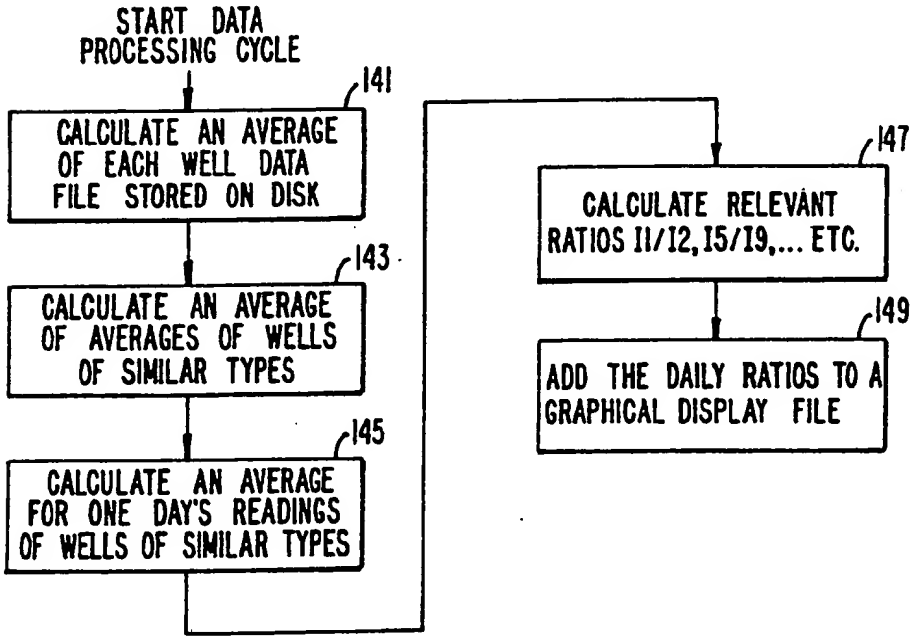
START DATA PROCESSING CYCLE
141
CALCULATE AN AVERAGE OF EACH WELL DATA FILE STORED ON DISK
143
CALCULATE AN AVERAGE OF AVERAGES OF WELLS OF SIMILAR TYPES
145
CALCULATE AN AVERAGE FOR ONE DAY'S READINGS OF WELLS OF SIMILAR TYPES
147
CALCULATE RELEVANT RATIOS I1/I2, I5/I9,... ETC.
149
ADD THE DAILY RATIOS TO A GRAPHICAL DISPLAY FILE

FIG. 6.

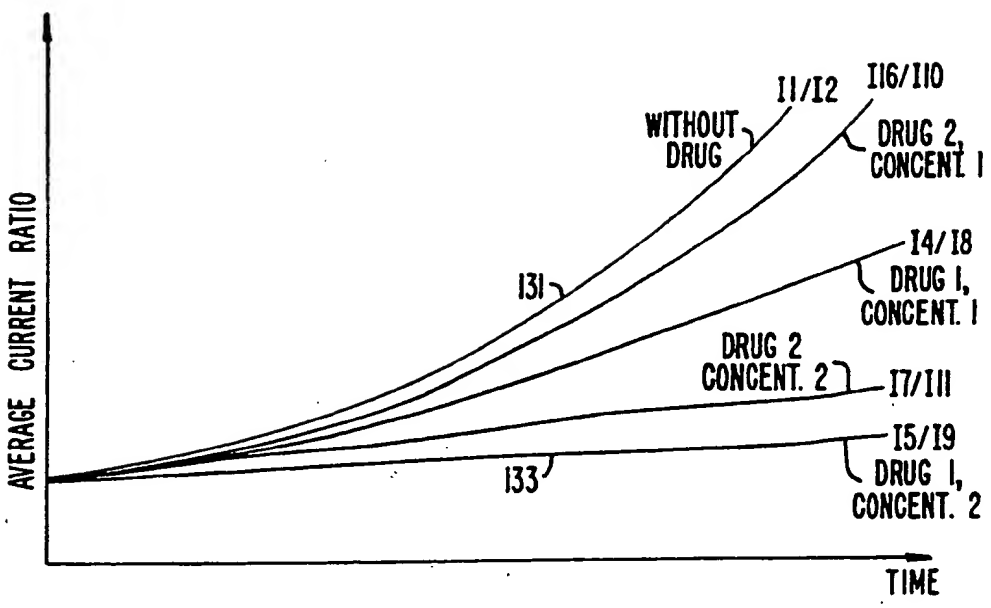

FIG. 7.

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application PCT/US91/02320

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C 12 Q 1/02

U.S. Cl: 435/29

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S. Cl.	435/29; 436/800; 435/240.2

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

MEDLINE " CANCER, SCREEN2, CHEMOTHERAP2" AU=KHAN, OMMAYA, ROSS

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
Y	US,A, 4,816,395, (HANCOCK ET AL.) 28 MARCH 1989, see paragraph spanning cols 1 and 2.	1-14
Y	DRUGS IN EXPERIMENTAL CLINICAL RESEARCH, VOLUME VII(5) ISSUED 1981, W.N. KHAN, "AN ELECTRICAL IMPEDANCE METHOD FOR RAPID MEASUREMENT OF TUMOR CELL SENSITIVITY TO ANTI-CANCER DRUGS", PAGES 641-647, see p.642, PARAGRAPHS SPANNING COLS 1 and 2.	1-16

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

16 MAY 1991

Date of Mailing of this International Search Report

25 JUN 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

JANE WILLIAMS